A Long-Term *In Vitro* Silicon-Based Microelectrode–Neuron Connection

WADE G. REGEHR, MEMBER, IEEE, JEROME PINE, AND DAVID B. RUTLEDGE, MEMBER, IEEE

Abstract—**A new method for long-term recording and stimulation applicable to cultured neurons has been developed. Silicon-based microelectrodes have been fabricated using integrated-circuit technology and micromachining. The chronic connection is made by positioning the tip of the "diving-board electrode" into contact with the top of the cell body. The electrode support structure is then glued to the bottom of the culture dish. Two-way electrical connections to *Helisoma* B19 neurons have been maintained for up to four days. This capability makes it possible to conduct experiments that are not practical using conventional techniques.**

I. Introduction

CELL-CULTURE techniques, in which neurons are grown in the artificial environment of a tissue culture dish, provide a powerful tool for answering fundamental neurobiological questions. Stimulating and recording from neurons is greatly simplified because the individual cells are visible and accessible, the biochemical environment is easily manipulated, and the nervous system can be greatly simplified. A number of interesting questions could be approached with techniques enabling a long-term, two-way electrical contact.

There are several ways to make electrical contact with a cell. A method that provides an excellent signal-to-noise ratio is inserting a glass electrode with a submicron tip into the cell. Another way to make electrical contact is a whole-cell patch to the cell [1]. A glass pipette a micron or more in diameter contacts the cell and a good seal is obtained by applying suction. It is then possible to break down the membrane beneath the patch and get inside the cell. However, these intracellular techniques have several disadvantages: it is difficult to maintain a connection for more than a few hours, establishing and maintaining a connection often damages the cell, and it is not generally practical to use more than two electrodes simultaneously.

Extracellular electrodes can also stimulate and record from cultured neurons. Cells have been grown on specially-prepared dishes with a multielectrode array pattern [2]–[6]. Such methods are noninvasive; they have been used successfully to stimulate neurons [4]–[6], and to record action potentials from cultured neurons for periods of weeks [3]. However, this technique is not sensitive to subthreshold synaptic signals. Furthermore, it may be difficult to interpret recordings since it is possible to record from several cells simultaneously, and a current pulse passed through an electrode may stimulate more than one cell.

It is also possible to stimulate and record by a loose-patch method [7] that does not break down the membrane [Fig. 1(a)]. This method is noninvasive, but it is still difficult to maintain the connection for long periods of time, and to communicate with more than two cells simultaneously. In special circumstances, it is possible to use multielectrode dishes in a manner similar to the loose-patch method by growing a neuron over an electrode in such a manner that a stable seal forms between the cell and the electrode [5], [8].

The electrode developed here is a silicon microdevice used in a manner similar to a loose-patch electrode [Fig. 1(b)]. Each electrode is manipulated into place and is then glued to the bottom of the tissue culture dish. A seal is made between the lower surface of the electrode and the cell in the same way that a seal is made to the bottom of a glass pipette, although suction cannot be applied when using this device. This establishes a one-to-one connection between a device and a cell for both stimulating and recording. A long flexible arm (the "diving board") makes gentle contact with the cell, while its silicon support pedestal is permanently mounted to the bottom of the culture dish. A gold strip sandwiched between two insulating layers leads to the cup-shaped structure at the end of the diving board. Here the diving board makes a seal to the neuron and electrical contact is made by a platinized gold electrode. An insulated gold wire extends vertically from the pedestal out of the solution to connect to the electronics. Diving-board electrodes can be successively positioned on several neurons of a culture, and then used for long-term multielectrode recording and stimulating.

II. Electrode Fabrication

Integrated-circuit technology and micromachining of silicon provide powerful tools to fabricate microdevices [9]. Such methods have the advantage of being well established for use in the semiconductor industry, and allow batch fabrication of many devices on one wafer with feature sizes down to a micron. The diving-board electrode is made by a five-mask process (Fig. 2). The first mask

Manuscript received February 29, 1988; revised June 22, 1988. This work was supported by NSF Grant BNS8603713.

W. G. Regehr was with the California Institute of Technology, Pasadena, CA 91125. He is now with the Moleculer Biophysics Research Department, AT&T Bell Laboratories, Murray Hill, NJ 07974.

J. Pine and D. B. Rutledge are with the California Institute of Technology, Pasadena, CA 91125.

IEEE Log Number 8823687.

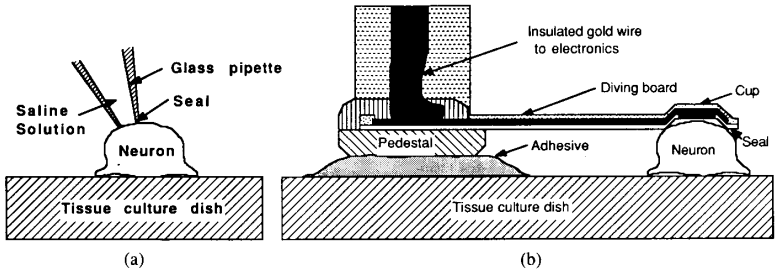

Fig. 1. (a) Schematic view of a conventional glass micropipette patch electrode being used to record from a cell. (b) Diving-board electrode in contact with a cell.

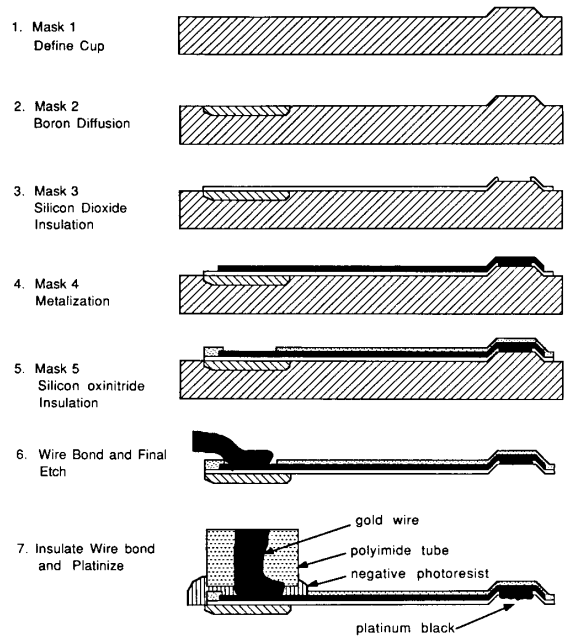

Fig. 2. Fabrication procedure for the diving-board electrode.

defines the cup structure. The outer rim of this cup forms the seal with the cell, and the cup must be deep enough to keep the metal surface away from the surface of the cell. The silicon is etched to a depth of 3 μm using an isotropic silicon etch 100 parts HNO_3, 100 parts CH_3COOH, and 15 parts HF [10]. Once this cup structure is defined it is necessary to use a thick photoresist with good step coverage; a 3 μm thick layer of Shipley 1400-37 photoresist is compatible with 3 μm cup structures and 2 μm line widths.

The second mask defines a pedestal to which the wire bond is attached, and which is glued to the bottom of the culture dish. While the pedestal should be mechanically strong, it cannot be thicker than a cell (10–50 μm). The height is determined by a boron etch-stop process [11]–[13]; therefore, working with very thin substrates is unnecessary. A 1 μm thick thermal-oxide layer is grown and then patterned with buffered HF to define the intended pedestal area. This is followed by boron diffusion for 10 h at 1170°C in a 5 percent O_2/95 percent N_2 atmosphere that heavily dopes the substrate to a concentration exceeding 5×10^{19} cm^{-3} to a depth of 10 μm. This defines the pedestal. The boron-glass layer on the surface of the wafer is removed by oxidizing for 1 h at 1100°C in dry O_2 and etching with buffered HF.

A 1000 Å thermal oxide layer is grown at 1000°C in steam for 10 min. The third mask defines the contact hole. In addition to being important for its insulating properties, this thin oxide is important because it touches the cell. This surface is a high-quality glass, which seems important for high-resistance seals comparable to those of patch pipettes.

Next 100 Å of chrome, 800 Å of gold, and 100 Å of chrome are successively evaporated on the wafer, and the fourth mask defines the metal pattern. The top layer of chrome is etched with Transene chromium mask etchant for 15 s; the gold is removed in 30 s in Transene TFA gold etchant diluted 1 : 4 with water [14]; followed by another 15 s in the chrome etch.

1 μm of silicon oxinitride is deposited by plasma enhanced chemical vapour deposition (PECVD) to form the top insulating layer [15], [16]. This layer determines the mechanical properties of the diving board, as well as defining tabs that later hold the electrodes in place to protect them from damage. The final mask patterns this top insulating layer, which is etched in a 85 percent CF_4/15 percent O_2 plasma [17]. A 7 μm thick layer of AZ4770 photoresist is used for this step to insure protection of the cap region during the plasma etch.

The wafer is scribed and broken into 4 × 20 mm pieces, each of which is glued to a piece of glass. A wire bond is made from a large gold bonding pad on the piece of glass to each of about 40 devices. The bonds are made of gold so that they will survive the next etching step. The wafer is then etched in an ethylenediamine, pyrocatechol, and water (EDP) solution [18], [19]. This etch attacks different silicon planes at different rates, and etches ⟨111⟩ planes very slowly. Furthermore, it does not attack regions that are doped with boron to levels in excess of 5×10^{19} cm^{-3}. After 3 h in EDP at 100°C, the undoped silicon is completely etched from beneath each electrode. Fig. 3 shows the device after the EDP etch. Each electrode is

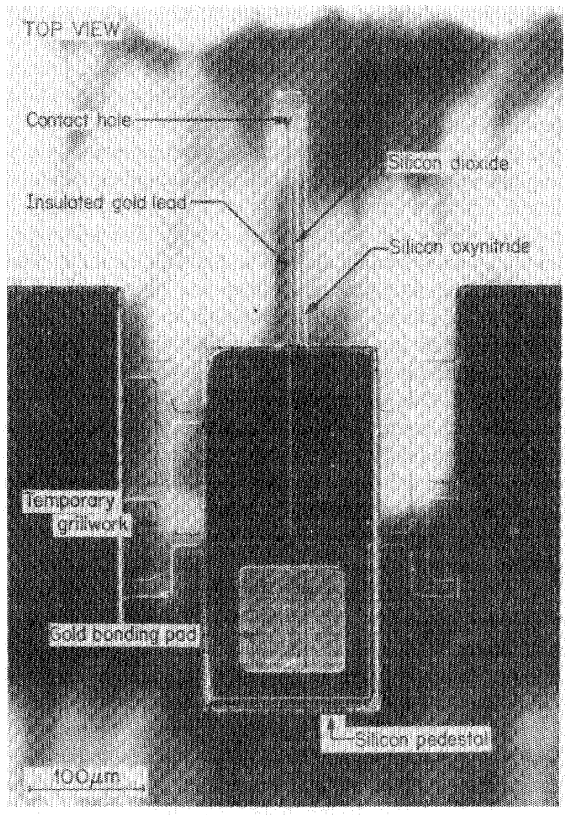

Fig. 3. Diving board electrode after removal from EDP. There would normally be a 25 μm diameter wire bonded to the gold pad at the back of the pedestal.

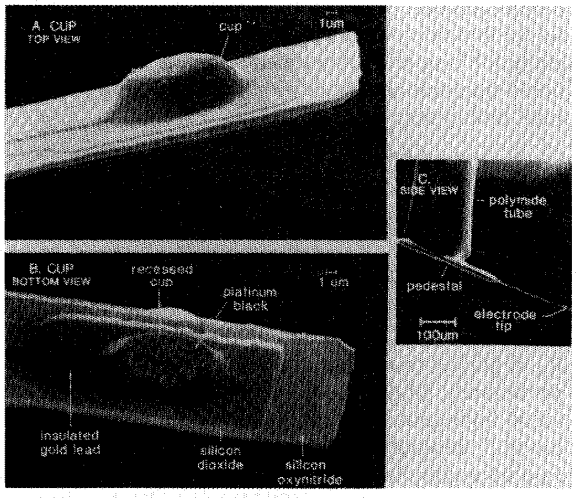

Fig. 4. A finished diving-board electrode. (a) Top view of the cup structure that is to fit above the neuron. (b) Bottom view of the cup structure. (c) Overview of the electrode.

temporarily held to the substrate by a silicon–oxinitride grill.

Each device is separated from the wafer and the wire bond is insulated. Each lead wire is manually loaded into a polyimide tube (Polymicro Technologies, Phoenix, AZ: i.d. 120 μm, o.d. 150 μm) and the wire bond is insulated by painting with negative photoresist. This polyimide tube can be held by tweezers and placed without compromising the insulation. The final step is to electroplate the electrode at the bottom of the diving board with platinum black, to produce a low-impedance contact to the electrolyte [20], [21]. By electroplating with a current of 2 nA (about 10 mA/cm^2) for 15 s the electrode impedance at 1 KHz drops from 25 MΩ to below 1 MΩ, while the platinum remains thin enough that it does not interfere with the seal.

Fig. 4 shows scanning electron micrographs of the cup structure, and an overview of a finished electrode. It takes an average of about 45 min to fabricate and test each device. Defective electrodes are eliminated on the basis of optical observations, and impedance measurements made before platinization. Satisfactory electrodes can be reused several times.

III. Mechanical Properties

In addition to considering the electrical properties of the diving-board electrode, it is necessary to take into account its mechanical properties. A relatively flat diving board is necessary: it enables simultaneous contact between the pedestal and the culture dish and between the electrode tip and the neuron and it makes the electrode exert a force directly downward on the cell, so the cell will not be pushed out from beneath the diving board.

However, there are stresses in each layer which may bend the diving board. This is illustrated in Fig. 5, which shows a cantilever of length l that is composed of two layers of thickness d_1 and d_2. The Young's modulus of the thick layer is given by Y_1 and the Poisson's ratio is ν_1. A stress σ in layer 2 bends the beam an amount δ. For small deflections and $d_1 >> d_2$ [22], we can write

$$\delta = \frac{3l^2(1 - \nu_1)\sigma d_2}{Y_1 d_1^2}. \quad (1)$$

In our electrodes, the thick layer is silicon oxinitride, 200 μm long and 1 μm thick. Young's modulus Y_1 1×10^{12} dyn/cm^2 [23] and the Poisson ratio ν_1 is 0.2. If $d_2 = 0.2$ μm a stress typical of insulating dielectrics of $\sigma = 5 \times 10^9$ dyn/cm^2, would cause an unacceptably large beam deflection of 100 μm. If the stress is compressive it will cause the cantilever to bend up as in the diagram, while if the stress is tensile the cantilever will bend down.

The present fabrication technique minimizes the deflection of the cantilever in two ways. First, the silicon-oxinitride layer is made very rigid compared to the other two layers by making it much thicker than the gold and the oxide layer, and by making the surface areas of the gold and the silicon dioxide layers small compared to the surface area of the oxinitride layer. Second, the stress of the deposited layers is empirically adjusted to minimize the beam deflection. It is difficult to change the stress in the gold and silicon-dioxide films in our electrodes, but

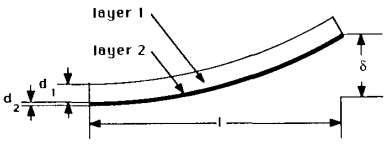

Fig. 5. Bending due to stress in a two-layer cantilever.

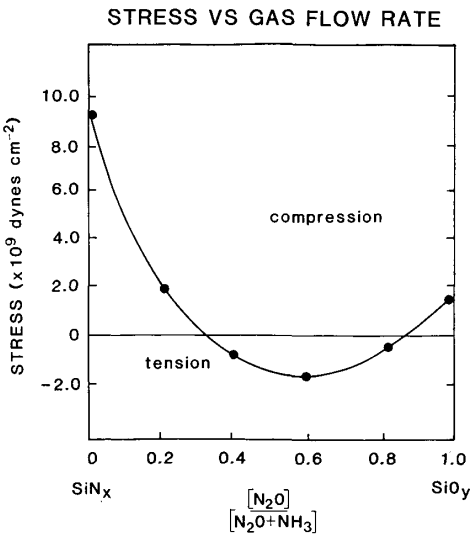

Fig. 6. Controlling the stress in oxinitride films by varying the flow rates of N_2O and NH_3 [23]. The films are deposited at 380°C on silicon. The x axis is the ratio of the N_2O flow rate to the total combined flow rate of N_2O and NH_3. When this ratio is 0 the film is silicon nitride, and when this ratio is 1 the film is silicon dioxide.

we can vary the stress in the silicon-oxynitride films by adjusting the ratio of oxygen to nitrogen in the film. This is done by varying the flow rates of N_2O and NH_3 during the PECVD process (Fig. 6). By depositing the silicon oxinitride layer at 380°C at a pressure of 1.5 torr, with flow rates of $NH_3/N_2O/SiH_4$ being 750/1500/250 sccm ($[N_2O]/[N_2O + NH_3] = 0.67$), the deposited layer had a tensile stress of 1.4×10^9 dyn/cm^2 and the resulting diving-board electrodes are flat to a few microns.

IV. Establishing the Chronic Connection

The diving-board electrodes are manipulated into place while being viewed through an inverted microscope equipped with phase and epifluorescence optics. The polyimide handle of an electrode is held by fine forceps mounted in a holder that provides pressure to hold the electrode firmly until it has been glued to the bottom of the culture dishes. Then the pressure is reduced and the electrode is gently released without damaging the glue joint. The holder is mounted in a special manipulator that in addition to the standard x, y, z degrees of freedom, provides θ and ϕ control needed to make the diving board flat relative to the surface of the tissue culture dish.

Once the electrode has been made flat relative to the culture dish, a metal needle is used to scratch the plastic where the pedestal is to be glued. A 50 μm diameter drop of glue is applied to the bottom of the tissue culture dish through a 10 μm diameter glass electrode using pressure to control the drop size. The glue is Electro-Lite Corporation (Danbury, CT) 4481 that sets in ultraviolet light and adheres well under water to all plastics tested, but not to glass or the polylysine or collagen coated substrates on which cells are grown. This is why the procedure of locally exposing the plastic is necessary. The adhesive is thinned with isopropyl alcohol so that it is easier to obtain small drops. After the glue drop has been applied, the electrode is placed with the pedestal in contact with the glue and the diving-board tip in contact with the cell. Once a seal resistance of several megohms has been obtained, the drop of glue is exposed to ultraviolet light from the mercury arc lamp of the epifluorescence illuminator. An iris is used to restrict the illumination to the glue drop and to prevent the cell from being exposed and possibly damaged. Based on physiological and morphological observations, the gluing process does not harm the cell, and the glue is nontoxic.

Electrical contact to the electrode is maintained during manipulation and gluing so that the seal resistance can be monitored. The culture dish has wires extending to the interior through holes in the side, and has a Ag/AgCl reference electrode mounted to the side of the dish. A flexible 25 μm gold wire bond is at the end of each wire. When the electrode has been positioned near the cell a conductive graphite paint TV tubecoat is used to connect the wire from the diving board electrode to one of the wires connected to the side of the dish, which are in turn connected to the external electronics. When the device has been glued it is possible to position the next diving-board electrode. When all the electrodes are mounted, the lid the dish is covered and the diving-board electrodes are ready for long-term experiments. Fig. 7 shows a diving-board electrode in contact with a cultured neuron from the snail *Helisoma*.

V. Stimulating and Recording with Diving-Board Electrodes

A. Equivalent Circuit

The operation of the diving-board electrode is very much like that of a loose-patch electrode [7]. Fig. 8(a) shows a schematic of an electrode in contact with a cell and an electrical equivalent circuit. The shunt resistance R_{sh} is greater than 1 GΩ and shunt capacitance C_{sh} is approximately 10 pF, so that for a low seal-resistance the shunt impedance can be ignored. The electrode impedance Z_e depends upon the type of electrode used. In the case of a glass loose-patch electrode it is essentially resistive and varies between 100 kΩ and 1 MΩ. For the metal diving-board electrode, the impedance Z_e varies as the area of the exposed metal and is primarily capacitive. It is desirable to have a low electrode-impedance for three reasons. First, it facilitates measuring the seal resistance. Second, a low impedance electrode reduces the Johnson noise and increases the signal-to-noise ratio of the neural

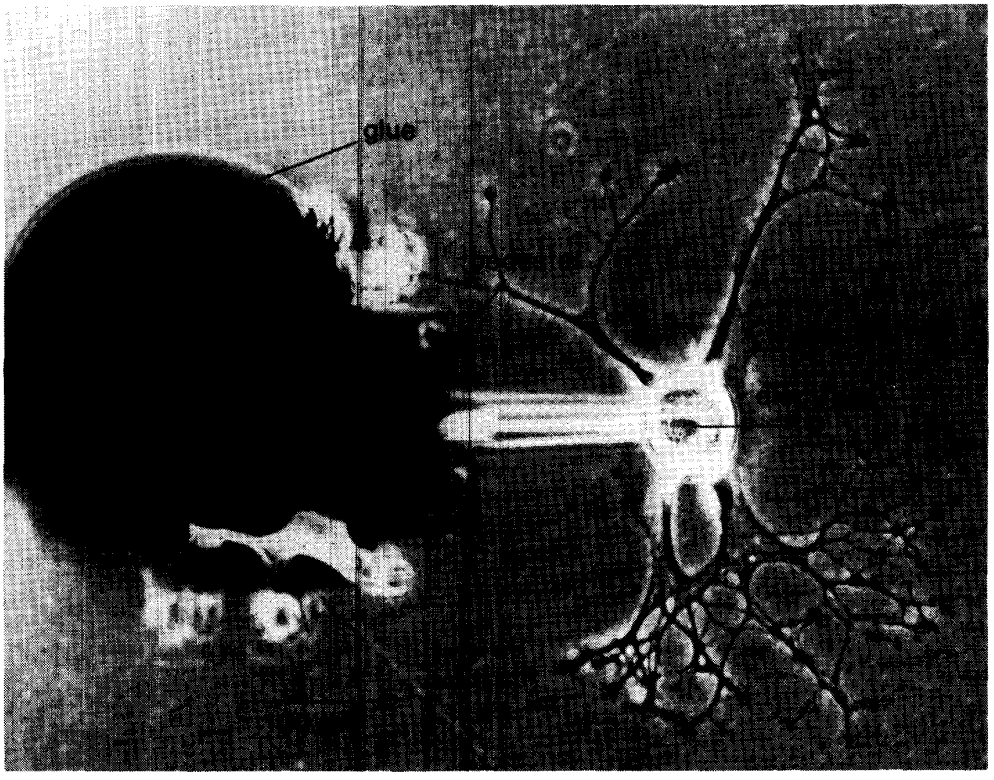

Fig. 7. Diving board electrode in chronic electrical contact with a *Helisoma* B19 neuron. The pedestal is glued to the bottom of the culture dish and the electrode tip is in contact with the cell body.

recordings. Third, a low impedance electrode helps avoid the problem of gas evolution when stimulating.

The patch region beneath the electrode in Fig. 8 is made up of a layer of membrane with a capacitance estimated by assuming a membrane capacitance of approximately 1 $\mu F/cm^2$ [25]; therefore, C_{m1} is 1 pF for a 100 μm^2 patch. Spanning this membrane are n different channel types with electrochemical driving forces $V_1 \cdots V_n$. The resistance $R_1 \cdots R_n$ corresponding to the each particular channel type are voltage and time dependent. The net resistance depends upon the channel densities, which is in general nonuniform over the cell and unknown in the patch region. The whole-cell impedance Z_{m2} is also voltage and time dependent. At the resting potential of the cell Z_{m2} can be approximated by a capacitor and a resistor in parallel. For *Helisoma* neurons B19 and B5, the resistance is typically about 50 MΩ and the capacitance 500 pF.

The seal resistance R_{seal} should be as large as possible. It is determined by the conductance of the thin film of tissue culture medium between the rim of the electrode and the cell. For *Helisoma*, neurons seals of 1–5 MΩ were obtained.

B. Recording

Fig. 8(b) is an equivalent circuit of a diving-board electrode being used to record from a neuron. $i_1 \cdots i_n$ are the currents passing through each particular type of channel, and i_c is the current through the membrane capacitance. The recorded voltage V_{out} is given by

$$V_{out} = \left(i_c + \sum_{chan=1}^{N} i_{chan} \right) R_{seal} \tag{2}$$

$$V_{out} = \left(C_{m1} \frac{dV_{cell}}{dt} + \sum_{chan=1}^{N} \frac{(V_{cell} - V_{chan})}{R_{chan}} \right) R_{seal} \tag{3}$$

$$V_{out} = V_{cap} + V_{ionic}. \tag{4}$$

This expression has two components. V_{cap} is the differentiated intracellular voltage seen through the capacitance of the membrane under the electrode. For this signal to be large, the action potential must be fast. For the rising phase of the action potential, the value of dV_{cell}/dt is approximately 100 mV/ms for *Helisoma* B19 neurons and 20 mV/ms for *Helisoma* B5 neurons. Assuming a membrane area of 100 μm^2, and a seal resistance of 2 MΩ for *Helisoma* B19 neurons, $V_{cap} \approx 200$ μV, and for *Helisoma* B5 neurons $V_{cap} \approx 40$ μV. V_{ionic} is due to ionic current flowing through channels open in the patch under the electrode. Signals recorded with the diving-board electrode have been primarily capacitive with the ionic component being small and variable in size.

The signal-to-noise ratio can be determined by considering the noise contributions. The primary source of noise is Johnson noise

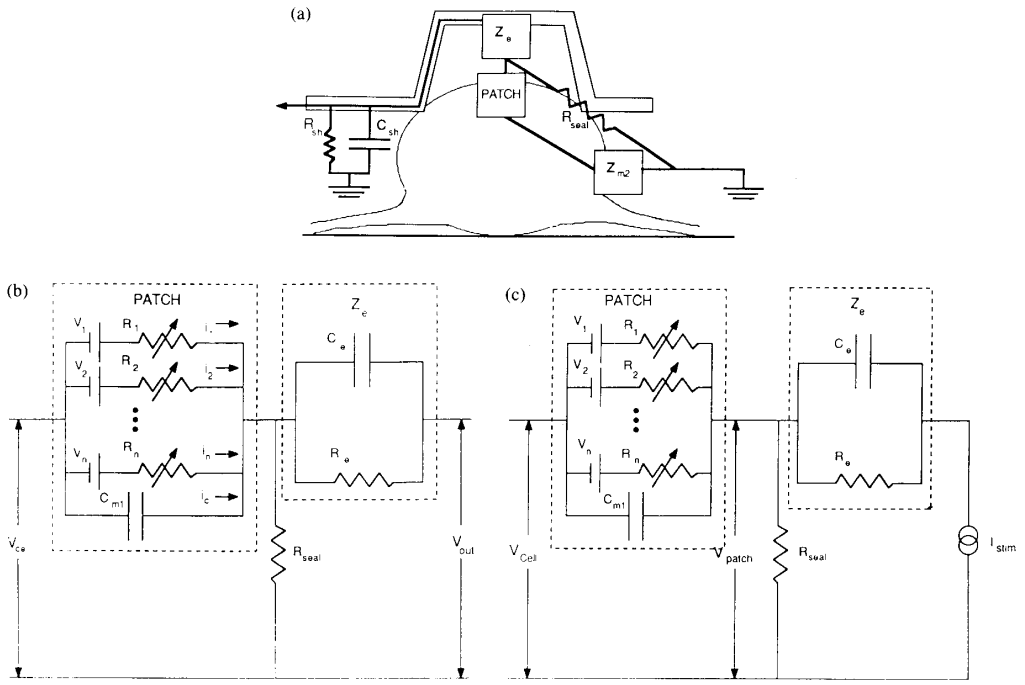

Fig. 8. (a) Schematic diagram of an electrode in contact with a cell and an approximate equivalent circuit for (b) recording and (c) stimulating.

$$V_{\text{noise}}(\text{rms}) = \sqrt{4(R_{\text{seal}} + R_e)\,kTB} \tag{5}$$

where the R_{seal} is the seal resistance, R_e is the real part of the electrode impedance, k is Boltzmann's constant, T is the temperature, and B is the bandwidth in hertz. At 300°C, for a 1 kHz bandwidth, a seal resistance of 2 MΩ, and an electrode with a real impedance R_e = 500 kΩ, the corresponding rms Johnson noise is 6 μV. Therefore, for R_{seal} = 2 MΩ, $V_{\text{cap}}/V_{\text{noise}}(\text{rms}) \approx 30$ for *Helisoma* B19 action potentials, and $V_{\text{cap}}/V_{\text{noise}}(\text{rms}) \approx 6$ for *Helisoma* B5 action potentials.

Recording subthreshold signals will be difficult since the signals are typically small and slow. Furthermore, signals are difficult to interpret since the intracellular signal is distorted by passing through the patch membrane. Very good seals are required in order to record subthreshold signals. If a gigaohm seal could be obtained, then it would be practical to electrically break down the cell membrane beneath the cup and have essentially a chronic whole-cell patch recording. Diving-board electrodes have been used in this manner, but so far the seal resistances have not been large enough to prevent a deterioration of the resting potential of the cell.

C. Stimulation

The equivalent circuit for stimulation is shown in Fig. 8(c). It is generally true that a membrane depolarization of approximately 15 mV will fire a neuron. As a current pulse is applied through the electrode, most of the current passes out beneath the cup, with a small fraction passing into the cell through the patch and then out through the rest of the cell. For a stimulus current pulse I_{stim}, the voltage applied to the patch is $V_{\text{patch}} = I_{\text{stim}} R_{\text{seal}}$ assuming $R_{\text{seal}} \ll Z_{m1}$. A positive current pulse thus hyperpolarizes the patch membrane, and depolarizes the rest of the cell membrane. For stimulus pulses short compared to the time constant of the cell (which is typically $\gtrsim$ 5 ms.), the internal voltage change is approximated by

$$\Delta V_{\text{cell}} = \frac{\Delta t(i)}{C_{m2}} \tag{6}$$

$$\Delta V_{\text{cell}} = \frac{\Delta t}{C_{m2}}\left(i_c + \sum_{\text{chan}=1}^{N} i_{\text{chan}}\right) \tag{7}$$

$$\Delta V_{\text{cell}} = \frac{\Delta t C_{m1}}{C_{m2}}\frac{dV_{\text{patch}}}{dt} + \frac{\Delta t}{C_{m2}}\left(\sum_{\text{chan}=1}^{N} \frac{(V_{\text{cell}} - V_{\text{patch}} - V_{\text{chan}})}{R_{\text{chan}}}\right). \tag{8}$$

Since channel type and density beneath the patch is unknown it is difficult to predict the required stimulus current.

Two constraints limit the amount of current that can be used for stimulating. First, if a voltage of greater than about 1 V is applied to a metal electrode, gas is evolved that kills the cells. An electrode can be approximated by a capacitor with a capacitance C_e. A current pulse with amplitude I_{stim} and duration Δt results in an electrode potential $\Delta V_e = I_{\text{stim}} \times \Delta t / C_e$ which limits the total charge $I\Delta t$ useful for stimulating. Second, I_{stim} must be kept small

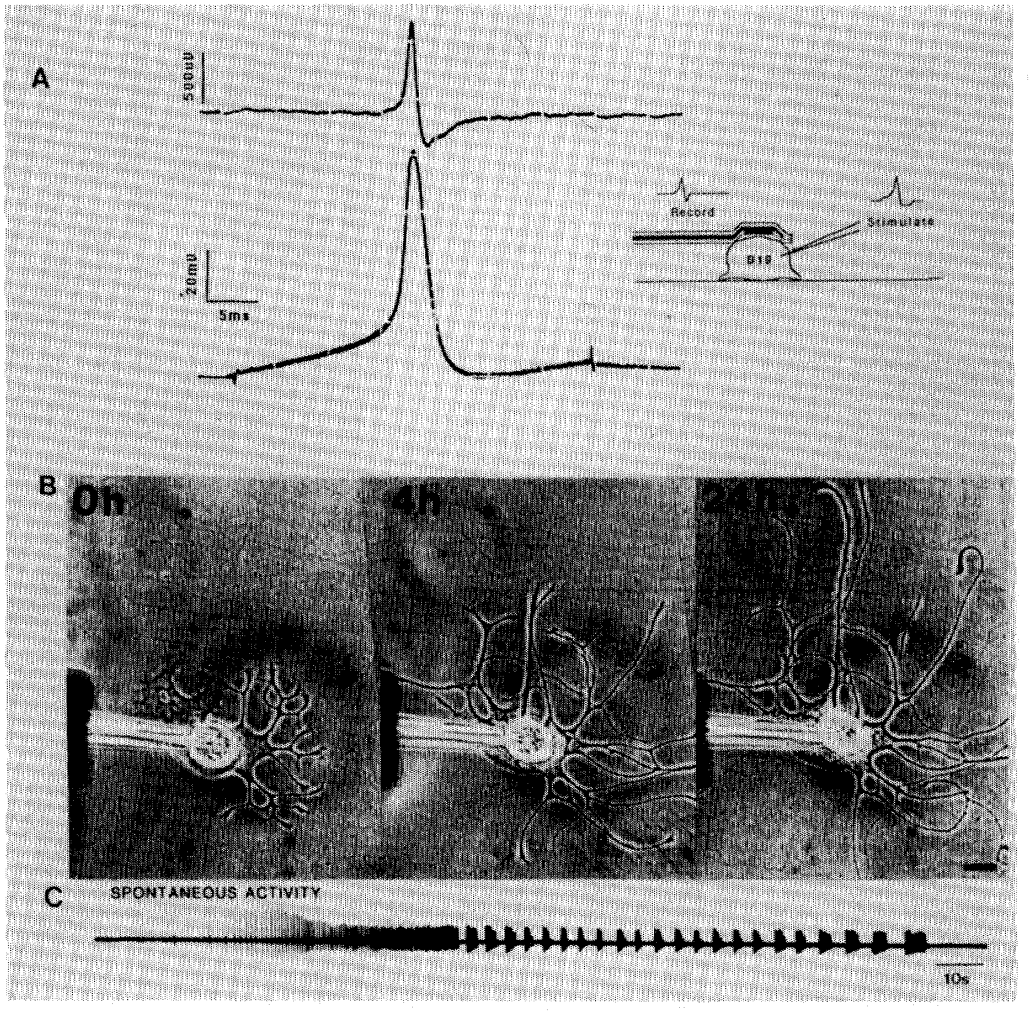

Fig. 9. (a) A *Helisoma* B19 neuron stimulated with a current pulse passed through an intracellular electrode, and the response of the cell recorded with the intracellular electrode, and the diving-board electrode (R_{seal} = 4.0 MΩ, bandwidth 10 Hz–1 KHz). (b) Successive pictures of a diving-board being used to record from a *Helisoma* B19 neuron at t = 0 h, t = 4 h, and t = 24 h. Calibration bar is 50 μm. (c) An example of spontaneous activity recorded at t = 2 hours (R_{seal} = 3 MΩ).

enough that V_{patch} remains less than about 300 mV or the membrane in the patch may be electrically broken down. This would damage the cell unless R_{seal} was much larger than the few megohms thus far achieved.

VI. Electrode Tests

Diving-board electrodes have been used primarily with identified *Helisoma* B5 and B19 neurons prepared in a manner that has been described elsewhere [26]. Two-electrode experiments were performed to understand device operation. In preliminary experiments, a 12 μm glass pipette was substituted for the diving-board electrode. Experiments were performed with an intracellular electrode and a patch pipette recording from the same cell. In this way, it was possible to compare the intracellular response with the response of the patch pipette. Once stimulation and recording were accomplished and understood with the patch pipette, a diving-board electrode was used. Other than the inherent electrical differences between liquid filled and metal electrodes, diving-board electrodes behave like glass patch-electrodes of the same tip diameter for both stimulation and recording.

A. Recording

It is possible to record action potentials with good signal-to-noise ratios with the seals obtained, but in order to record subthreshold signals, the seal resistance needs to be greatly improved. In the experiment of Fig. 9 with the diving-board electrode mounted on top of a neuron, an intracellular electrode was used to stimulate using a current pulse and the diving-board electrode was used to record the resulting action potential. Signal-to-noise ratios

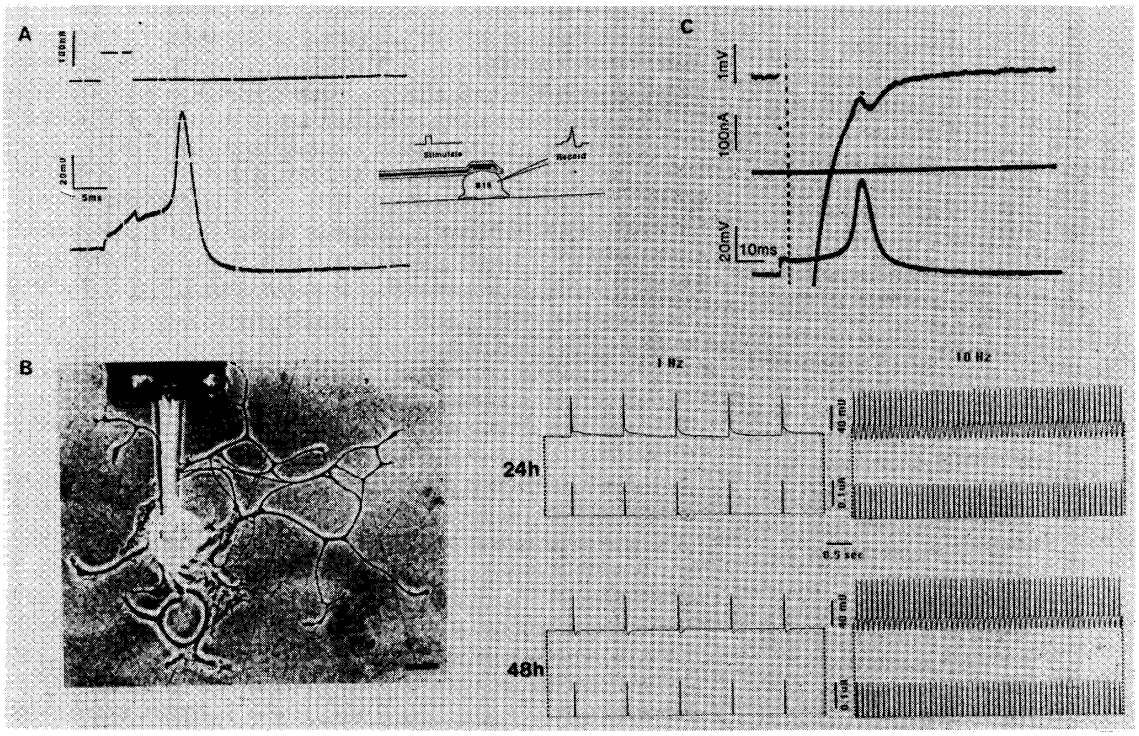

Fig. 10. (a) Stimulation of a *Helisoma* B19 neuron using a current pulse passed through a diving-board electrode to stimulate and recording using an intracellular electrode. (I_{stim} = 100 nA, Δt = 5 ms, V_{patch} = 210 mV) (b) A diving-board electrode in electrical contact with a *Helisoma* B19 neuron. Calibration bar is 50 μm (I_{stim} = 140 nA, Δt = 0.5 ms, V_{patch} = 170 mV). 24 h after the device was placed on the neuron an intracellular electrode was used to monitor the activity of the cell and current pulses passed through the electrode were used to stimulate the cell at 1 Hz and at 10 Hz. The stimulus current passed through the diving-board electrode is shown below the intracellular voltage record. The intracellular electrode was then removed while the diving board electrode continued to stimulate the cell at 1 Hz. At time t = 48 h an intracellular electrode was used to verify that the stimulus threshold had not changed. (c) A *Helisoma* neuron B19 stimulated by a diving-board electrode (I_{stim} = 100 nA, Δt = 0.2 ms, V_{patch} = 210 mV). Since the stimulus is near threshold the resulting action potential is many milliseconds after the stimulus pulse and it can be recorded on the diving-board electrode. (R_{seal} = 1.5 MΩ, bandwidth 100 Hz-1 KHz).

for action potentials recorded from *Helisoma* B19 neurons were typically 20–100 : 1, and for *Helisoma* B5 neurons 4–10 : 1.

Diving-board electrodes have been used to record spontaneous activity from *Helisoma* B19 neurons for up to four days. Fig. 9(b) shows a diving-board electrode that was being used to monitor the spontaneous activity of a *Helisoma* B19 neuron for two days. Pictures at t = 0 h, t = 4 h, and t = 24 h show normal cell growth. Fig. 9(c) is an example of the spontaneous activity recorded from this cell.

B. Stimulation

Diving-board electrodes have been used to stimulate *Helisoma* neurons. In the experiment of Fig. 10(a), a current pulse from a diving-board electrode was used to stimulate the neurons while an intracellular electrode was used to monitor the potential of the cell. *Helisoma* cells have been stimulated intermittently for up to four days, at which time the experiment was terminated. Fig. 10(b) shows a diving-board electrode that was placed on a cell at time t = 0 h. At 24 h, the cell was penetrated with an intracellular electrode to monitor the activity of the cell. Then the current pulses from the diving board and stimulated the cell was at 1 Hz and 10 Hz. The intracellular electrode was then removed and the cell was stimulated at 1 Hz for 24 h. At that time, the cell was again penetrated to verify that the cell was still being stimulated and that the threshold had not changed.

It is necessary to measure the evoked action potential to verify that the cell is being stimulated. While simultaneous intracellular recording has been used, a less invasive method is preferable. The most desirable situation is to measure the evoked action potential with the same diving-board electrode used to stimulate the cell. The primary difficulty is the large stimulus artifact which is on the order of 200 mV, much larger than the recorded action potential, which is on the order of 100 μV. There are two ways of solving this problem. The first is to increase the stimulus pulse very slowly; at threshold a late action potential is recorded many milliseconds after the stimulus pulse. Once threshold has been determined, the stimulus

pulse can be increased by 10 percent to obtain reliable stimulation. In Fig. 10(c), *Helisoma* B19 is stimulated by a diving-board electrode and the response of the late action potential is recorded by the same electrode. The second solution, which has not been done yet, is to remove the stimulus artifact using a combination of digital and analog techniques [7].

C. Preliminary Studies on Vertebrate Neurons

Preliminary studies have also been performed with dissociated rat superior cervical ganglion neurons (SCG neurons) prepared using conventional techniques [27]. There are several factors that make working with SCG neurons more difficult than with *Helisoma* neurons: they are smaller (a diameter of about 25 μm for SCG neurons compared to about 50 μm for *Helisoma* neurons), the extracellular solution resistivity is lower by about a factor of three resulting in lower seal resistances, and SCG neurons are in general more delicate than *Helisoma* neurons. Despite these difficulties, it has been possible to stimulate SCG neurons, and to record action potentials with a signal-to-noise ratio of 10:1 (R_{seal} = 0.4 MΩ). Long-term stimulation and recording studies will be performed in the near future. The success of the diving-board electrodes on SCG neurons shows their versatility and indicates that they can be used with neurons 20 μm in diameter and longer. The devices can be scaled down for use with smaller neurons.

Conclusion

A new method has been developed to establish a chronic two-way, neuron-microdevice connection. It is a very good extracellular connection that is maintained without the need for a manipulator. It has the associated advantage of being noninvasive but with poor sensitivity for subthreshold events. Many experiments can be performed using the device in its present state. For example, the activity-dependence of neurite outgrowth [28] could be studied more extensively using this device which facilitates noninvasive stimulation for longer times than are possible using conventional techniques, and spontaneous activity in cultured neurons can be chronically and noninvasively monitored. This is not possible using conventional techniques. There is also the future possibility of improving the seal resistance and obtaining a chronic connection similar to an intracellular connection.

Acknowledgment

The authors are grateful to Dr. S. B. Kater for supplying *Helisoma* neurons and for making his laboratory available for experiments, and to Dr. C. Cohan for helping with the *Helisoma* physiology.

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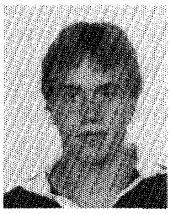
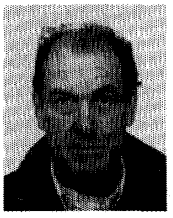
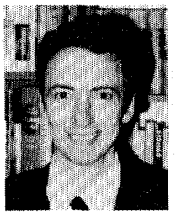


Wade G. Regehr (S'87-M'88) was born in Shaunavon, Sask., Canada, on December 23, 1960. He received the B.S. degree in physics from the University of Regina, Regina, Sask., in 1982 and the M.S. and Ph.D. degrees in applied physics from the California Institute of Technology, Pasadena, CA, in 1984 and 1988, respectively. His research is in long-term, two-way connections to neurons in cell culture. He was supported in the graduate work by an NSERC Canada 1967 scholarship.



Jerome Pine received the Ph.D. degree in physics from Cornell University, Ithaca, NY, in 1956.

He is Professor of Physics at the California Institute of Technology, Pasadena, CA, and his research is in neural networks and primary school science education.



David B. Rutledge (S'75-M'80) was born in Savannah, GA, on January 12, 1952. He received the B.A. degree in mathematics from Williams College, Williamstown, MA, in 1973, the M.A. degree in electrical sciences from Cambridge University in 1975, and the Ph.D. degree in electrical engineering from the University of California, Berkeley, in 1980.

In 1980 he joined the faculty at the California Institute of Technology, Pasadena, CA, where he is now Associate Professor of Electrical Engineering. His research is in developing millimeter and submillimeter-wave monolithic integrated circuits and applications, and in software for computer-aided design and measurement.

Dr. Rutledge received the IBM Faculty Development Award in 1983 and the NSF Presidential Young Investigator Award in 1984.